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Date

Kane Koo

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

FRESCO, J., et al.

Serial No.: 09/341,079

Art Unit: 1631

Filed on: 02 July 1999

Examiner: Stephen Siu

For: **STABILIZATION OF TRIPLEXES
BY WATER STRUCTURE-MAKING
SUBSTANCES**

To The Honorable
Assistant Commissioner of Patents
Washington, D.C. 20231

DECLARATION OF JACQUES R. FRESCO

I, Jacques R. Fresco, declare that:

1. All statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

2. I received an A.B. degree in Biology and Chemistry in 1947 from New York University; an M.S. degree in Biology in 1949 from New York University; a Ph.D. degree in Biochemistry in 1953 from New York University; and took postdoctoral training in nucleic acid chemistry at the Sloan-Kettering Institute, New York City from 1952-54 and at the Department of Chemistry of Harvard University, Cambridge, MA from 1956-1960; and that,

3. From 1960 to the present, I have been a Professor at Princeton University, Princeton, New Jersey (from 1990 to the present in the Department of Molecular Biology) with the title of Pfeiffer Professor in the Life Sciences from 1977 to the present; and that,

4. I, together with John Laurence Richard Lavelle, am a joint inventor of the invention of the present patent application; and that,

5. The present invention demonstrates an unexpected increase in the effectiveness and efficiency of stabilizing nucleic acid triplexes when compared to previous methods, primarily through the use of compounds/substances which demonstrate a water structure-making property which impacts on the solvation of the DNA; and that

6. I have read and understood the papers of Kim et al., *Biochemistry* 35, 1187-1194 (1996); Robles et al., *Journal American Chemistry Society*, 118, 5820-5821 (1996); Kiyama et al. *Nucleic Acids Research*, 23, 452-458 (1995); D'Souza et al. *Bioorganic and Medicinal Chemistry Letters*, 4, 965-970 (1994) and Shimizu et al., *Biochemistry* 33, 606-613 (1994), references cited by the U.S. Patent Office Official Action; and that,

7. None of those cited references disclose or suggest increasing the effectiveness or efficiency of third strand binding and triplex stability by using water structure-making substances; and that,

8. The stabilization described in the Kim et al. article does not stabilize third strand binding by using water structure-making substances but, instead uses 9-aminoacridine and bis-

acridine to stabilize third strand binding via intercalation of these substances between base triplets of a triplex; and that,

9. The method disclosed in the Robles et al. article does not disclose the poly(ethylene glycol) of the present invention. The hexa(ethylene glycol) used by Robles et al. does not make use of water structure-making properties of glycols; rather the glycol used by Robles et al. is covalently linked to the third strand and presumably stabilizes third strand binding by virtue of minor groove insertion. Moreover, the glycols used in Robles et al. actually weaken third strand binding when they are not tethered to the third strand. The Robles et al. method requires radically different concentrations of the "stabilizing" substance when compared with the concentrations of the poly(ethylene glycol) used in the present invention; and that,

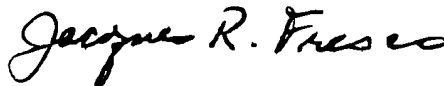
10. The work of Kiyama and Oishi discloses that a single modest concentration of cetyltrimethylammoniumbromide can protect the duplex region between two oligonucleotide-linked triplex segments from digestion by a restriction enzyme, a methylase and DNase. It is inferred that the protection is due to the stabilization of the two triplex segments but no direct evidence for this inference is presented. Possible alternative mechanisms for this activity include, for example, facilitating non-sequence-specific interaction of the single strand linker with the non-Pu-Pyr segment of the duplex, possibly due to positive charges on the bound CTAB moieties. This mechanism, however, cannot explain the stabilization of triplexes by cationic detergents stemming from their water structure-making activity as in the present invention. Much higher concentrations of detergent, than are used by Kiyama and Oishi, are required for triplex stabilization in the present invention, and the degree of triplex stabilization is strongly dependent on the cationic detergent concentration; and that,

11. The D'Souza and Kool article describes their observations that ethanol destabilizes triple helices in which a circle containing two pyrimidine sequence elements bind to a homopurine single strand sequence. They ascribe this behavior to the enhanced interstrand charge repulsion due to the effect of ethanol on the dielectric constant of the medium. This effect of ethanol is opposite to the ethanol solvent effect of the present invention. Moreover, D'Souza and Kool do not teach of third strand binding, since such was known and patented

before their work; further, in showing that use of ethanol destabilizes third strand binding, they make no mention of ethanol or any other water structure-making substances that stabilize triplexes; and that

12. The Shimizu et al. reference discloses enhancement of third strand binding stability by using particular base analogs as part of the third strand itself; these effects are not related to any water structure-making ability of the solution additives of the present invention. Moreover, the base analogs Shimizu et al. studied did not significantly enhance triplex stability over those formed using canonical bases; and that,

Respectfully submitted,



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